

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Kelli E. Smith and Richard Weinshank

U.S. Serial No.: Not Yet Known

Filed : Herewith

For : DNA Encoding A Human Receptor (hp15a) And
Uses Thereof

1185 Avenue of the Americas
New York, New York 10036
December 19, 2001

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT AND INFORMATION DISCLOSURE STATEMENT

Please amend the subject application as follows:

In the Specification:

On page 1, line 5, please insert the following as a separate paragraph:

--This application is a continuation-in-part of U.S. Serial No. 09/179,798, filed October 27, 1998, the contents of which are herein incorporated by reference.--

Please replace the paragraph beginning on page 70, line 7 through page 71, line 26, with the following paragraph:

--Methods for recording currents in Xenopus oocytes

Female *Xenopus laevis* (*Xenopus-1*, Ann Arbor, MI) are anesthetized in 0.2% tricain (3-aminobenzoic acid ethyl ester, Sigma Chemical

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Corp.) and a portion of ovary is removed using aseptic technique (Quick and Lester, 1994). Oocytes are defolliculated using 2 mg/ml collagenase (Worthington Biochemical Corp., Freehold, NJ) in a solution containing 87.5 mM NaCl, 2 mM KCl, 2 mM MgCl₂, and 5 mM HEPES, pH 7.5. Oocytes may be injected (Nanoject, Drummond Scientific, Broomall, PA) with mammalian mRNA. Other oocytes may be injected with a mixture of mammalian mRNA and mRNA encoding the genes for G-protein-activated inward rectifiers (GIRK1 and GIRK4, U.S. Patent Nos. 5,734,021 and 5,728,535). Genes encoding G-protein inwardly rectifying K⁺ (GIRK) channels 1 and 4 (GIRK1 and GIRK4) were obtained by PCR using the published sequences (Kubo et al., 1993; Dascal et al., 1993; Krapivinsky et al., 1995 and 1995b) to derive appropriate 5' and 3' primers. Human heart cDNA was used as template together with the primers 5'-CGCGGATCCATTATGTCTGCCTCCGAAGGAAATTTG-3' (SEQ ID NO: 11) and 5'-CGCGAATTCTTATGTGAAGCGATCAGAGTTCATTTTC -3' (SEQ ID NO: 12) for GIRK1 and 5'-GCGGGATCCGCTATGGCTGGTGATTCTAGGAATG-3' (SEQ ID NO: 13) and 5'- CCGGAATTCCCCTCACACCGAGCCCCCTGG-3' (SEQ ID NO: 14) for GIRK4. In each primer pair, the upstream primer contained a BamHI site and the downstream primer contained an EcoRI site to facilitate cloning of the PCR product into pcDNA1-Amp (Invitrogen). The transcription template for the mammalian receptor may be similarly obtained. mRNAs are prepared from separate DNA plasmids containing the complete coding regions of the mammalian receptor, GIRK1, and GIRK4. Plasmids are linearized and transcribed using the T7 polymerase ("Message Machine", Ambion). Alternatively, mRNA may be translated from a template generated by PCR, incorporating a T7 promoter and a poly A⁺ tail. Each oocyte receives 2 ng each of GIRK1 and GIRK4 mRNA in combination with 25 ng of mammalian receptor mRNA. After injection of mRNA, oocytes are incubated at 16° C on a rotating platform for 3-8 days. Dual electrode voltage clamp ("GeneClamp", Axon

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Instruments Inc., Foster City, CA) is performed using 3 M KCl-filled glass microelectrodes having resistances of 1-3 Mohms. Unless otherwise specified, oocytes are voltage clamped at a holding potential of -80 mV. During recordings, oocytes are bathed in continuously flowing (2-5 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, and 5 mM HEPES, pH 7.5 ("ND96"), or, in the case of oocytes expressing GIRK1 and GIRK4, elevated K⁺ containing 96 mM KCl, 2 mM NaCl, 2 mM CaCl₂, 2 mM MgCl₂, and 5 mM HEPES, pH 7.5 ("hK"). Drugs are applied by switching from a series of gravity fed perfusion lines.--

Please replace the paragraph beginning on page 74, line 25, with the following paragraph:

--RT-PCR

For the detection of low levels of RNA encoding hp-15a receptor, RT-PCR was carried out on mRNA extracted from human tissue. Reverse transcription and PCR reactions were carried out in 50 µl volumes using EzrTth DNA polymerase. Primers with the following sequences were used:

RA hp15F24

ACCTCACACTGGCTGATCTCCTCT (SEQ ID NO: 15)--

Please replace the paragraph beginning on page 74, line 35, with the following paragraph:

--RA hp15B1

GTAGATGCCCATGAGGATGGTGGTG (SEQ ID NO: 16)--

On page 75, line 24, please insert the following new paragraphs:

--Quantitative PCR

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Quantitative PCR using a fluorogenic probe with real time detection: Quantitative PCR using fluorogenic probes used to characterize the distribution of SNORF49 RNA. This assay utilizes two oligonucleotides for conventional PCR amplification and a third specific oligonucleotide probe that is labeled with a reporter at the 5' end and a quencher at the 3' end of the oligonucleotide. In the instant invention, FAM (6-carboxyfluorescein) was used as the reporter, and BH1 (Biosearch) was used as the quencher. As amplification progresses, the labeled oligonucleotide probe hybridizes to the gene sequence between the two oligonucleotides used for amplification. The nuclease activity of *Taq* thermostable DNA polymerase is utilized to cleave the labeled probe. This separates the quencher from the reporter and generates a fluorescent signal that is directly proportional to the amount of amplicon generated. This labeled probe confers a high degree of specificity. Non-specific amplification is not detected as the labeled probe does not hybridize and as a consequence is not cleaved. All experiments were conducted in a PE7700 Sequence Detection System (PE Biosystems, Foster City CA),

Quantitative RT-PCR: Quantitative RT-PCR was used for the detection of hp15a RNA.

For use as a template in quantitative PCR reactions, cDNA was synthesized by reverse transcription from total human RNA. Reverse transcription by SuperScriptII RNase H⁻ and (GibcoBRL/life Technologies) was primed using random hexamers. Parallel reactions included ³²P labeled dCTP to allow quantification of the cDNA. Following reverse transcription, cDNA was phenol/chloroform extracted and precipitated. Incorporation of ³²P dCTP was assessed after precipitation with trichloroacetic acid and the amount of cDNA synthesized was calculated.

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For PCR reactions, primers with the following oligonucleotide sequences were used:

Forward primer hp15a-663F had a sequence which began with 5'-C located at nucleotide number 723 and ended at with G-3' at nucleotide number 742. (See Figures 1A-1B.)

Reverse primer hp15a-727R had a sequence complimentary to the sequence which began at 5'-C located at nucleotide number 769 and ending with A-3' at nucleotide number 787. (See Figures 1A-1B.)

Fluorogenic oligonucleotide probe hp15a-684T had a sequence which began with 5'(6-FAM)-C located at nucleotide number 744 and ended at with G-(TAMRA)3' at nucleotide number 767. (See Figures 1A-1B.)

Using this primer set, amplicon length is 65 bp for hp15a. Each PCR reaction contained 3.0 ng cDNA. Oligonucleotide concentrations were: 500 nM of forward and reverse primers, and 200 nM of fluorogenic probe. PCR reactions were carried out in 50 ml volumes using TaqMan universal PCR master mix (PE Applied Biosystems). Buffer for RT-PCR reactions contained a fluor used as a passive reference (ROX: Perkin Elmer proprietary passive reference I). All reagents for PCR (except cDNA and oligonucleotide primers) were obtained from Perkin Elmer (Foster City, CA). Reactions were carried in a PE7700 sequence detection system (PE Applied Biosystems) using the following thermal cycler profile: 50EC 2 min., 95EC 10 min., followed by 40 cycles of: 95EC, 15 sec., 60EC 1 min.

Standard curves for quantification of human hp15a were constructed using genomic DNA. Negative controls consisted of

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mRNA blanks, as well as primer and mRNA blanks. To confirm that the mRNA was not contaminated with genomic DNA, PCR reactions were carried out without reverse transcription using Taq DNA polymerase. Integrity of RNA was assessed by amplification of RNA coding for cyclophilin or glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Following reverse transcription and PCR amplification, data was analyzed using PE Biosystems sequence detection software. The fluorescent signal from each well was normalized using an internal passive reference, and data was fitted a standard curve to obtain relative quantities of hp15a expression.--

On page 78, line 30, after Table 1, please insert the following new paragraphs:

--Quantitative PCR

Detection of mRNA coding for human hp15a receptor: mRNA was isolated from multiple tissues (Table 2) and assayed by quantitative PCR methods as described hereinabove.

High levels of mRNA encoding hp15a receptor in lung, with relatively lower expression in most of the other regions assayed, implicates this receptor in respiratory disorders and asthma.

In addition to the potential therapeutic applications identified in Table 2, the localization data for mRNA encoding the human hp15a receptor indicates that the DNA encoding the human hp15a receptor can be used to predict the likelihood that a tissue sample of unknown tissue origin is of lung origin with respect to a given individual. In addition, with respect to a given individual, one could determine whether a given tissue sample of unknown origin is of lung origin as opposed to having the origin of another tissue, e.g. the heart, pancreas, or liver. Such

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determinations may be used for various purposes including but not limited to the detection of tumor metastasis.

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Table 2

Distribution of mRNA coding for human hp15a receptor using qRT-PCR

mRNA encoding hp15a is expressed as copies/ng cDNA

Region	hp15a	Potential therapeutic applications
adrenal gland (whole)	114	Regulation of metabolic steroids, regulation of epinephrine release
amygdala	245	Depression, phobias, anxiety, mood disorders
cerebellum	88	Motor coordination
cerebral cortex	121	Cognition, sensory and motor integration
dorsal root ganglia	267	Sensory transmission
heart	13	Cardiovascular disorders
hippocampus	206	Cognition/memory
hypothalamus	206	Appetite/ obesity, neuroendocrine regulation
kidney cortex	39	Electrolyte balance, hypertension
kidney medulla	116	Electrolyte balance, hypertension
liver	14	Diabetes
lung	5114	Respiratory disorders, asthma
medulla	189	Sensory transmission
pancreas	13	Diabetes, endocrine disorders
pituitary (whole)	56	Endocrine/ neuroendocrine regulation
pontine reticular formation	74	Regulation of somatosensory, motor, visual, auditory, autonomic and affective processes
skeletal muscle	61	Musculoskeletal disorders
small intestine	63	Gastrointestinal disorders
spinal cord lumbar	614	Analgesia, sensory modulation and transmission
spleen	528	Immune disorders
stomach	76	Gastrointestinal disorders

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testes	576	Reproductive function, regulation of steroid hormones
thalamus	46	Sensory integration disorders
uterus	89	Reproductive function, regulation of steroid hormones

--
Please delete the Sequence Listing on pages 83-86 and replace it with the Sequence Listing attached hereto as **Exhibit 1**.

Attached hereto as **Exhibit 2** is a marked-up version of the amended text showing the changes made.

In the Claims:

Please cancel claims 1-144 without disclaimer or prejudice to applicants' right to pursue the subject matter of this claim in a future continuation or divisional application.

Please add new claims 145-146 as follows:

--145. (New) A recombinant nucleic acid comprising consecutive nucleotides encoding a human hp15a receptor, wherein the human hp15a receptor has an amino acid sequence identical to the sequence of the human hp15a receptor encoded by the nucleotide sequence beginning at the start codon at positions 61-63 and ending at the stop codon at positions 1249-1251 as indicated in Figures 1A-1B (SEQ ID NO: 1).--

--146. (New) A recombinant nucleic acid comprising consecutive nucleotides encoding a human hp15a receptor, wherein the nucleotides have a sequence

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identical to the sequence of the human hp15a receptor-encoding nucleotides contained in plasmid hp15a (ATCC Accession No. 209447).--

REMARKS

Claims 1-144 were pending in the subject application. By this Preliminary Amendment, applicants have canceled claims 1-144 without prejudice or disclaimer, and added new claims 145-146. Accordingly, upon entry of this Preliminary Amendment, claims 145-146 will be pending and under examination.

Applicants have amended the specification to provide a Sequence Listing for the sequences disclosed in the application. Applicants have also amended the specification to correct the numbering of SEQ ID Nos to conform with the Sequence Listing. Applicants maintain that these amendments raise no issue of new matter and are fully supported by the application as filed.

Applicants maintain that the addition of claims 145-146 raises no issue of new matter and is fully supported by the specification as filed.

Support for new claim 145 may be found inter alia in the specification, as originally-filed, on page 19, lines 5-8; page 16, lines 3-8; and Figures 1A-1B. Support for new claim 146 may be found inter alia in the specification, as originally-filed, on page 23, lines 5-10.

Accordingly, applicants respectfully request that this Amendment be entered.

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Sequence Listing

The Sequence Listing in the subject application is identical to that filed with the parent of the subject application, U.S. Serial No. 09/179,798, filed October 27, 1998. Applicants are filing as part of the subject application copies of the paper copy of the Sequence Listing (**Exhibit 1**) and Statement in Accordance With 37 C.F.R. §1.821(f) (**Exhibit 3**) which were filed with U.S. Serial No. 09/179,798, on June 28, 2000. The computer readable form in the subject application is identical to that filed in U.S. Serial No. 09/179,798 on June 28, 2000. In Accordance with 37 C.F.R. §1.821(e), please use the computer readable form filed in U.S. Serial No. 09/179,798 on June 28, 2000 as the computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the sequence listing that will be used for the instant application.

Information Disclosure Statement

In accordance with their duty of disclosure under 37 C.F.R. §1.56, applicants would like to direct the Examiner's attention to the following reference which is listed on the attached Form PTO-1449 (**Exhibit 4**) and attached hereto as **Exhibit 5**:

1. Yousefi, et al., "Cloning and expression analysis of a novel G-protein-coupled receptor selectively expressed on granulocytes," *J. Leukoc. Biol.* (June 2001) **69(6)**: 1045-52.

Applicants would also like to direct the Examiner's attention to the following references which are listed on the attached Form

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PTO-1449 (**Exhibit 4**) and were previously cited in connection with the prosecution of U.S. Serial No. 09/179,798, filed October 27, 1998; the subject application claims priority under 35 U.S.C. §120 of the filing date of that application. According to 37 C.F.R. §1.98(d), copies of patents or publications that were previously cited by, or submitted to, the Patent Office in connection with such prior applications need not accompany the Information Disclosure Statement. Accordingly, copies of the following references are not attached to this Information Disclosure Statement:

1. European Patent Application EP 0 853 125 A2, published July 15, 1998.
2. Hodgson, J., "Receptor Screening and the Search for New Pharmaceuticals," *Bio/Technology* (1992), **10**: 973-977;
3. Murdoch, C. et al., "Chemokine receptors and their role in inflammation and infectious diseases," *Blood*, (2000) **95(10)**: 3032-3043;
4. Rudiger, J., "Characteristics of the amino acids as components of a peptide hormone sequence," *Peptide Hormones* (1976), pages 1-7; and
5. Watson, S. et al., "The G-Protein Linked Receptor Facts Book", *Academic Press*, (1994) pages 2-6 and 223-230.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned

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attorney invites the Examiner to telephone him at the number provided below.

No fee, other than the enclosed \$370.00 fee for filing the subject application, is deemed necessary in connection with the filing of this Preliminary Amendment. However, if an additional fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,



John P. White
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(212) 278-0400

Marked-up Version of Amendments

Additions to the text are indicated by double underlining; deletions are indicated by square brackets.

In the Specification:

The replacement paragraph on page 70, line 7 through page 71, line 26:

Methods for recording currents in Xenopus oocytes

Female *Xenopus laevis* (*Xenopus-1*, Ann Arbor, MI) are anesthetized in 0.2% tricain (3-aminobenzoic acid ethyl ester, Sigma Chemical Corp.) and a portion of ovary is removed using aseptic technique (Quick and Lester, 1994). Oocytes are defolliculated using 2 mg/ml collagenase (Worthington Biochemical Corp., Freehold, NJ) in a solution containing 87.5 mM NaCl, 2 mM KCl, 2 mM MgCl₂ and 5 mM HEPES, pH 7.5. Oocytes may be injected (Nanoject, Drummond Scientific, Broomall, PA) with mammalian mRNA. Other oocytes may be injected with a mixture of mammalian mRNA and mRNA encoding the genes for G-protein-activated inward rectifiers (GIRK1 and GIRK4, U.S. Patent Nos. 5,734,021 and 5,728,535). Genes encoding G-protein inwardly rectifying K⁺ (GIRK) channels 1 and 4 (GIRK1 and GIRK4) were obtained by PCR using the published sequences (Kubo et al., 1993; Dascal et al., 1993; Krapivinsky et al., 1995 and 1995b) to derive appropriate 5' and 3' primers. Human heart cDNA was used as template together with the primers

5'-CGCGGATCCATTATGTCTGCACTCCGAAGGAAATTG-3' [(Seq. I.D. No. 15)]
(SEQ ID NO: 11) and

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Exhibit 2

5'-CGCGAATTCTTATGTGAAGCGATCAGAGTCATTTTC -3' [(Seq. I.D. No. 16)]

(SEQ ID NO: 12) for GIRK1 and

5'-GCGGGATCCGCTATGGCTGGTGATTCTAGGAATG-3' [(Seq. I.D. No. 17)] (SEQ

ID NO: 13) and

5'- CCGGAATTCCCCTCACACCGAGCCCCCTGG-3' [(Seq. I.D. No. 18)] (SEQ ID

NO: 14) for GIRK4.

In each primer pair, the upstream primer contained a BamHI site and the downstream primer contained an EcoRI site to facilitate cloning of the PCR product into pcDNA1-Amp (Invitrogen). The transcription template for the mammalian receptor may be similarly obtained. mRNAs are prepared from separate DNA plasmids containing the complete coding regions of the mammalian receptor, GIRK1, and GIRK4. Plasmids are linearized and transcribed using the T7 polymerase ("Message Machine", Ambion). Alternatively, mRNA may be translated from a template generated by PCR, incorporating a T7 promoter and a poly A⁺ tail. Each oocyte receives 2 ng each of GIRK1 and GIRK4 mRNA in combination with 25 ng of mammalian receptor mRNA. After injection of mRNA, oocytes are incubated at 16° C on a rotating platform for 3-8 days. Dual electrode voltage clamp ("GeneClamp", Axon Instruments Inc., Foster City, CA) is performed using 3 M KCl-filled glass microelectrodes having resistances of 1-3 Mohms. Unless otherwise specified, oocytes are voltage clamped at a holding potential of -80 mV. During recordings, oocytes are bathed in continuously flowing (2-5 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, and 5 mM HEPES, pH 7.5 ("ND96"), or, in the case of oocytes expressing GIRK1 and GIRK4, elevated K⁺ containing 96 mM KCl, 2 mM NaCl, 2 mM CaCl₂, 2 mM MgCl₂, and 5 mM HEPES, pH 7.5 ("hK"). Drugs are applied by switching from a series of gravity fed perfusion lines.

The replacement paragraph on page 74, line 25:

RT-PCR

For the detection of low levels of RNA encoding hp-15a receptor, RT-PCR was carried out on mRNA extracted from human tissue. Reverse transcription and PCR reactions were carried out in 50 µl volumes using EzrTth DNA polymerase. Primers with the following sequences were used:

RA hp15F24

ACCTCACACTGGCTGATCTCCTCT [(Seq. I.D. No. 19)] (SEQ ID NO: 15)

The replacement paragraph on page 74, line 35:

RA hp15B1

GTAGATGCCCATGAGGATGGTGGTG [(Seq. I.D. No. 20)] (SEQ ID NO: 16)